Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Parasitology 125 (2004) 163–181
veterinary parasitology
www.elsevier.com/locate/vetpar

Factors that influence the prevalence of acaricide resistance and tick-borne diseases

L.D. Foil [a,*], P. Coleman [b], M. Eisler [c], H. Fragoso-Sanchez [d], Z. Garcia-Vazquez [e], F.D. Guerrero [f], N.N. Jonsson [g], I.G. Langstaff [g], A.Y. Li [f], N. Machila [c], R.J. Miller [h], J. Morton [i], J.H. Pruett [f], S. Torr [i]

[a] *Entomology Department, 402 Life Sciences Building, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA*
[b] *London School of Hygiene and Tropical Medicine, London, UK*
[c] *Centre for Tropical Veterinary Medicine, Edinburgh, UK*
[d] *Centro Nacional de Servicios de Constatación en Salud Animal, Carretera Federal Cuernavaca Cuautla, Km 11.5, Col. Progreso Jiutepec, Morelos, Mexico*
[e] *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, CENID-PAVET, Apartado Postal 206, CIVAC, C.P. 62550, Morelos, Mexico*
[f] *USDA-ARS Knipling-Bushland US Livestock Insects Research Laboratory, 2700 Fredricksburg Road, Kerrville, TX 78028, USA*
[g] *School of Veterinary Science, University of Queensland, Qld 4072, Australia*
[h] *USDA-ARS Cattle Fever Tick Research Laboratory, Mission, TX, USA*
[i] *Natural Resources Institute, Chatham, UK*

Abstract

This manuscript provides a summary of the results presented at a symposium organized to accumulate information on factors that influence the prevalence of acaricide resistance and tick-borne diseases. This symposium was part of the 19th International Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP), held in New Orleans, LA, USA, during August 10–14, 2003. Populations of southern cattle ticks, *Boophilus microplus*, from Mexico have developed resistance to many classes of acaricide including chlorinated hydrocarbons (DDT), pyrethroids, organophosphates, and formamidines (amitraz). Target site mutations are the most common resistance mechanism observed, but there are examples of metabolic mechanisms. In many pyrethroid resistant strains, a single target site mutation on the Na^+ channel confers very high resistance (resistance ratios: >1000×) to both DDT and all pyrethroid acaricides. Acetylcholine esterase affinity for OPs is changed in resistant tick populations. A second mechanism of OP resistance is linked to cytochrome P450 monooxygenase activity. A PCR-based assay to detect a

* Corresponding author. Tel.: +1 225 578 1825; fax: +1 225 578 1643.
E-mail address: lfoil@agctr.lsu.edu (L.D. Foil).

0304-4017/$ – see front matter
doi:10.1016/j.vetpar.2004.05.012

specific sodium channel gene mutation that is associated with resistance to permethrin has been developed. This assay can be performed on individual ticks at any life stage with results available in a few hours. A number of Mexican strains of *B. microplus* with varying profiles of pesticide resistance have been genotyped using this test. Additionally, a specific metabolic esterase with permethrin-hydrolyzing activity, CzEst9, has been purified and its gene coding region cloned. This esterase has been associated with high resistance to permethrin in one Mexican tick population. Work is continuing to clone specific acetylcholinesterase (AChE) and carboxylesterase genes that appear to be involved in resistance to organophosphates. Our ultimate goal is the design of a battery of DNA- or ELISA-based assays capable of rapidly genotyping individual ticks to obtain a comprehensive profile of their susceptibility to various pesticides. More outbreaks of clinical bovine babesisois and anaplasmosis have been associated with the presence of synthetic pyrethroid (SP) resistance when compared to OP and amidine resistance. This may be the result of differences in the temporal and geographic patterns of resistance development to the different acaricides. If acaricide resistance develops slowly, herd immunity may not be affected. The use of pesticides for the control of pests of cattle other than ticks can affect the incidence of tick resistance and tick-borne diseases. Simple analytical models of tick- and tsetse-borne diseases suggest that reducing the abundance of ticks, by treating cattle with pyrethroids for example, can have a variety of effects on tick-borne diseases. In the worst-case scenario, the models suggest that treating cattle might not only have no impact on trypanosomosis but could increase the incidence of tick-borne disease. In the best-case, treatment could reduce the incidence of both trypanosomosis and tick-borne diseases Surveys of beef and dairy properties in Queensland for which tick resistance to amitraz was known were intended to provide a clear understanding of the economic and management consequences resistance had on their properties. Farmers continued to use amitraz as the major acaricide for tick control after the diagnosis of resistance, although it was supplemented with moxidectin (dairy farms) or fluazuron, macrocyclic lactones or cypermethrin/chlorfenvinphos.

Keywords: Tick control; *Boophilus microplus*; Acaricide resistance; Bovine babesiosis; Tick-borne diseases

1. Introduction

Since the eradication of *Boophilus* ticks from the US, several new classes of acaricides have been developed, but resistance in tick populations has been described for the majority of these products. One objective of this symposium was to compile information on the distribution of, and types of acaricide resistance in tick populations. We also were interested in determining if use of pesticides to control cattle pests other than ticks is influencing the occurrence of acaricide resistance in tick populations.

The concept of enzootic stability allows an understanding of how immunization of young cattle by the natural vectors of disease agents could result in herd immunity and reduce incidence of disease. Since Texas cattle do not have immunity to Texas cattle fever, a second objective of this symposium was to consider whether resistant ticks could penetrate into tick-free areas in the US; and if so, what type of diagnostic techniques could be used to rapidly identify resistant mechanisms being used.

2. Materials and methods

2.1. Mechanisms of resistance and molecular techniques

There were common laboratory methods for determining resistance levels and mechanisms among the different studies. Protocols for rearing ticks, performing bioassays, isolating DNA and performing the PCR assays are described or referenced in Guerrero et al. (2002), while the permethrin hydrolytic assay is described in Jamroz et al. (2000). The Food and Agriculture larval packet test (LPT) was used to measure the level of acaricide susceptibility in populations of *B. microplus* from Mexico (FAO, 1984). Synergists were used in the LPT procedure to disable specific enzymatic systems that may confer resistance to a certain acaricide.

2.2. Methods of tsetse control

The only sure way of controlling trypanosomosis in the long term is by eradicating tsetse from a region, as illustrated by tsetse control programs conducted successfully in Botswana, Nigeria, South Africa and Zimbabwe (Jordan, 1986). These relied on the large-scale application of insecticides to the resting sites of tsetse, by either large numbers of spray men applying a persistent insecticide such as DDT, or repeated aerial application of insecticides such as endosulfan. These national successes are small in comparison to the continent's ∼10 million square kilometers of tsetse-infested territory. Moreover, the prospect of large-scale aerial- and ground-spraying operations being undertaken in the near future are poor; government funding and institutional capacity to undertake such operations have largely disappeared and these technologies are beyond the capacities of even the richest livestock keepers.

Ground- and aerial-spraying aims to kill all the flies in an area after they emerge and before they manage to deposit a larva in the ground, with control being applied until all the flies in the ground have emerged. An alternative strategy is to apply a small, but sustained level of mortality to a tsetse population over a longer period by attracting tsetse to lethal baits. The baits may be either artificial devices, such as traps or insecticide-treated targets baited with synthetic host odors, or natural baits such as cattle treated with pyrethroids. The low reproductive rate of tsetse means that a low density (e.g. 4 targets/km^2) of evenly-spaced artificial baits can eradicate tsetse populations within two years (Vale, 1993; Willemse, 1991; Dransfield et al., 1990; Thompson et al., 1991).

In contrast to ground- and aerial-spraying, bait technologies can be implemented by livestock owners and they provide at least some measure of protection against re-invasion (Hargrove, 2000). Consequently, the large-scale operations of the past have been replaced by small (∼500–1000 km^2) operations implemented and funded, at least in part, by livestock-owning communities. These operations aim to reduce disease incidence rather than eliminate the disease entirely.

2.3. Survey of producers

Survey techniques were used to determine the impact of amitraz resistance in Australia. A personal telephone questionnaire was administered to a sample of all the dairy and beef

cattle producers in Queensland who submitted ticks that were diagnosed with amitraz resistance between 1981 and 2003. One hundred distinct cattle properties were identified with amitraz resistance between 1981 and 2003, comprising 56 dairy and 44 beef enterprises. Amitraz resistance was recorded as either Ulam or Ultimo strain and included nine suspect Ultimo detections (by laboratory definition), and 19 suspect Ulam strain detections. Ultimo strain demonstrates resistance to amtiraz and all synthetic pyrethroids, while Ulam strain demonstrates resistance to amitraz in the absence of resistance to all of the synthetic pyrethroids. Producers whose record showed a suspect resistance status were questioned to confirm that there had been evidence of dipping failure with amitraz at the time of resistance diagnosis. One producer reported no dipping failure and was excluded from the survey.

The questionnaire was constructed to determine how amitraz resistance had affected cattle farming operations and to provide empirical data to support or negate field observations that amitraz might regain usefulness after detection of resistance. Additional questions were intended to measure the level of participant motivation for the interview by using open questions about their level of concern regarding cattle ticks and interest in research investigations, and by inviting speculation on likely sources of amitraz resistant tick populations.

3. Results and discussion

3.1. Mechanisms of resistance

Our studies have shown that most recent collections of resistant ticks from Mexico are resistant to more than one class of acaricide. In studies by Li et al. (2003, 2004) of OP resistance, all strains that had a resistance ratio of 3.0 and higher to coumaphos were also resistant to diazinon. The synergist piperonyl butoxide (PBO) synergized the toxicity in all of these strains except for the San Roman. This suggests that resistance to OPs is at least in part the result of cytochrome P450 detoxification (*cytP450*) of coroxon and diazoxon. Pruett (2002) showed that an insensitive acetylcholine esterase was involved in OP resistance in at least two of the strains tested, the San Roman and Caporal.

At least two different mechanisms were also found to confer resistance to pyrethroids. Miller et al. (1999) found that two populations were resistant to the pyrethoid acaricides. In addition, these populations were shown to be resistant to DDT. Synergists did not increase the susceptibility of these populations. Target sight mediated resistance was confirmed by He et al. (1999) who discovered a mutation on the Na^+ channel. Guerrero et al. (2001) developed this into a PCR test.

In the second pyrethroid resistance there was no resistance to DDT-triphenylphosphate (TTP) synergized pyrethroid toxicity in this population which suggested that a metabolic mechanism was responsible. Jamroz et al. (2000) confirmed this with discovery that CzEst9 esterase activity was much higher in this population.

Formamidine resistance is the least understood. Triphenylphosphate was shown to synergize amitraz toxicity 6-fold, whereas in the susceptible population, TPP only synergized amitraz toxicity 2-fold. This suggested that esterase plays some role in amitraz resistance. However, because the resistance ratio averages over 50, another mechanism such as a target site mutation, confers resistance.

Table 1
Phenotypes of various acaricide resistant tick populations from Mexico[a]

Strain	Permethrin[b]		Coumaphos[b]	
	Phenotype	Resistance ratio	Phenotype	Resistance ratio
Tuxpan	SUS[c]	1.6	RES[c]	6.8
Coatzacoalcos	RES	250	RES	3.6
San Felipe	RES	1840	SUS	1.4
Corrales	RES	6900	SUS	1.3

[a] See Miller et al., 1999.
[b] Relative to Gonzalez control susceptible strain resistance ratio = 1.
[c] SUS: susceptible; RES: resistance.

3.2. Molecular techniques

Four acaricide resistant Mexican tick populations were analyzed by larval packet bioassays (Table 1) and Tuxpan was found to be pyrethroid susceptible and OP resistant, Coatzacoalcos pyrethroid and OP resistant, and San Felipe and Corrales were pyrethroid resistant and OP susceptible. The analysis by the two PCR mutation detection assays and the HPLC permethrin hydrolysis assay are shown in Table 2 and could have been accomplished in approximately 3 days. In many arthropod species, mutations in the sodium channel have been directly associated with pyrethroid resistance. Thus, the high level of homozygous mutated larvae (RR) in both the San Felipe (71%) and Corrales (97%) populations indicate a major component of their pyrethroid resistance is due to mutated sodium channel target site. It is arguable that other mechanisms of pyrethroid resistance might be occurring in these two strains and the sodium channel PCR assay only detects a single mechanism. However, with a great majority of the individuals possessing the pyrethroid insensitive form of the sodium channel, it is likely that the presence of this mechanism in this level within the population is sufficient to lead to control failure. There were no homozygous susceptible sodium channel alleles (SS) detected in either the San Felipe or Corrales populations.

Table 2
Molecular assays for pyrethroid resistance in various tick populations from Mexico

Strain	Sodium channel[a]			CzEst9[a]			Hydrolysis[b]	Mechanism[c]
	SS	SR	RR	SS	SR	RR	Relative activity	
Gonzalez	94	3	3	79	18	3	1	Sus
Tuxpan	100	0	0	25	53	22	1.8	Sus
Coatzacoalcos	92	8	0	0	0	100	5.3	Met
San Felipe	0	29	71	26	47	27	(Not done)	TS
Corrales	0	3	97	36	47	17	1.5	TS

[a] Percentage of population with genotypes as: SS, homozygous pyrethroid susceptible; SR, heterozygous; RR, homozygous pyrethroid resistant.
[b] Relative to Gonzalez control susceptible strain permethrin hydrolytic activity = 1.
[c] TS, target site; Met: metabolic esterase; Sus: susceptible.

Field efficacy trials to correlate control failure with % pyrethroid insensitive sodium channel alleles would be very helpful. In fact, there has been little published information to guide predictions of field efficacy based on the larval packet test, another shortcoming of that bioassay. Particularly in populations which are just beginning to develop resistance, one cannot use LC_{50} values for predictions of efficacy or when to expect control failure in a specific population. The larval packet test does not identify resistance mechanism unless synergists, which are not completely specific in mode of action, are included as part of the bioassay procedure. Gene specific assays, such as the sodium channel PCR assay, is unequivocal in determining a mechanism. This sodium channel assay of the San Felipe and Corrales populations clearly showed that target site resistance is present in most of the individuals, which will render pyrethroid pesticides ineffective, as they rely on sodium channel function disruption for their toxicity. It is possible that other sodium channel mutations could arise independently which would not be detected by the specific PCR assay reported here. However, several pyrethroid resistant populations of *B. microplus* have been surveyed for mutated sodium channel genes and only the single amino acid changing mutation which the PCR assay is designed to detect, has been found (He et al., 1999; Jamroz et al., 2000). Another aspect of this PCR assay is that it can be performed on any life stage of the tick, even samples preserved in alcohol or dry ice, with results available in a single day if necessary. Obviously, the larval packet test requires at least one live gravid female tick, facilities to induce egg deposition and hatch, properly rear larvae, handle pesticides and perform the necessary statistical analysis. The PCR assay can also be performed on a tick hemolymph sample drawn from a live tick, which can then be used for further studies or propagation if desired.

The CzEst9 permethrin-hydrolyzing esterase identified by Jamroz et al. (2000) as possessing elevated activity in the Cz strain and purified and characterized by Pruett et al. (2002) appears to facilitate pyrethroid resistance through a mechanism involving overexpression of the esterase. Despite the presence of a mutant *CzEst9* allele which was reported to be most prevalent in the Cz strain possessing the elevated CzEst9 activity, later studies showed the mutation seemed to provide only an incremental, though statistically significant, amount of additional pyrethroid resistance compared to the wild type allele (Guerrero et al., 2002). Thus, the *CzEst9* mutation-detecting PCR assay is not as informative as the sodium channel PCR assay regarding decisions on pesticide resistance and tick control issues. The Cz strain was reported to possess elevated *CzEst9* copy number (Hernandez et al., 2000). Hernandez et al. (2002) reported at least a 5-fold increase in *CzEst9* transcript in the Cz strain compared to other strains with different susceptibilities to pyrethroid. Pruett et al. (2002) reported indirect evidence that CzEst9 protein is more prevalent in the Cz strain than a susceptible control. Thus, an assay to specifically quantitate CzEst9 protein activity in tick populations seems most appropriate and would present another rapid molecular based assay for determination of a pyrethroid resistance mechanism.

Since the US border dipping vats are charged with OP, the issue of OP resistance in Mexico is of utmost importance to maintaining the US free of *Boophilus*. A study of OP kinetics in OP resistant *B. microplus* from Mexico identified populations with reduced AChE activity relative to susceptible populations (Pruett, 2002), indicating target site resistance. We have purified an enzyme from OP resistant *B. microplus* with AChE-like activity and are in the

process of obtaining amino acid sequence information to facilitate cloning the coding region from OP susceptible and resistant ticks and searching for resistance-associated amino acid differences. Two reports have presented putative AChE gene coding regions (Baxter and Barker, 1998; Hernandez et al., 1999), however, they possessed low sequence homology to each other and the proteins encoded by these genes were not expressed to confirm AChE-like activity. Additionally, there was no OP resistance-associated amino acid differences found between susceptible and resistant ticks in the coding regions of either of these putative AChEs. It is possible that neither of these genes code for an AChE that is involved in OP resistance or that target site OP resistance is due to transcript or translational product modifications which would not be reflected in the amino acid sequence of the protein. With this uncertainty, it is not possible to predict what type of molecular assay for target site OP resistance might emerge.

3.3. Acaricide resistance and enzootic stability

In endemic tick areas where cattle are raised, young animals are infected with bovine babesiosis without clinical signs; herd immunity is established due to continued reinfection with *Babesia* spp. In the tropical areas of Mexico, *B. microplus* infestations are high and the farmers have to treat animals every 21 days, which in some cases produces enzootic instability (Fig. 1). When *B. microplus* tick populations are controlled to low levels of tick infestation, some bovine babesiosis outbreaks have occurred due to the presence of the enzootic instability (Benavides, 1985). With low tick infestation (0.2 ticks/animal per day) in natural or artificial conditions, the proportion of animals at risk to become infected and show clinical babesiosis is 11%. If the number of ticks per animal increases, the risk of clinical cases of bovine babesiosis decreases since the herd immunity also is increased. It has been reported that for a herd with an average of 2 ticks/animal per day, 29% are protected, 49% are at risk to show clinical babesiosis and 22% will not become infected by *Babesia* spp. until 4 years of age (Benavides, 1985). The level of tick control and corresponding herd immunity can be affected by acaricide resistance; examples of the levels of resistance that occur are provided in Tables 3 and 4.

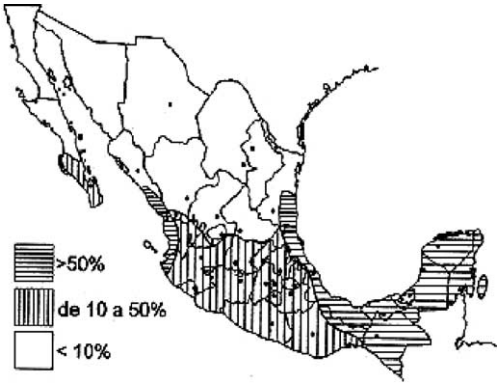

Fig. 1. Seroprevalence of bovine babesiosis distribution in Mexico.

Table 3
Efficacy of acaricides in *B. microplus* susceptible and organophosphate resistant ticks by adult immersion test

Compound, concentration	Control (%)		
	Susceptible	Tuxpan[a]	Tempoal[b]
Coumaphos, 200 ppm	97.94	51.97	22.01
Chlorfenvinphos, 300 ppm	99.99	97.60	98.00
Chlorpyriphos, 300 ppm	99.71	97.28	94.89
Ethion, 560 ppm	99.77	33.99	–
Diazinón	99.65	21.27	12.06
Lindano	99.92	99.30	22.03
Flumethrin, 40 ppm	100	100	100
Cypermethrin, 150 ppm	92.76	97.21	94.87
Amitraz, 150 ppm	100	100	100
Deltamethrin, 20 ppm	100	100	100

[a] Resistant to coumaphos.
[b] Resistant to organochlorinate.

3.4. Impact of tsetse control on tick-borne diseases

The convenience and relatively low cost of treating cattle with insecticide has led to a particularly marked rise in the use of pyrethroid-treated cattle for tsetse control. These insecticide formulations were originally developed for controlling ticks, and thus by attempting to control tsetse, many farmers who previously have not controlled ticks are now doing so inadvertently. Theoretical considerations suggest that this may disrupt enzootic stability for tick-borne diseases.

In Burkina Faso, Bauer et al. (1992) treated 2000 cattle with flumethrin at monthly intervals and observed a >95% reduction in tsetse numbers, and a decrease in the prevalence of trypanosomosis from 40 to 7% compared to a rise from 16 to 32% in a neighboring untreated area. They also observed that the burdens of *Ambylomma*, *Boophilus* and *Hyalomma* within the treated area were 70–90% less than those in the untreated area.

A tsetse control operation in Tanzania provides an example of a tsetse control operation unexpectedly reducing the prevalence of tick-borne disease. At Mkwaja ranch, Fox

Table 4
Efficacy of acaricides in *B. microplus* susceptible and pyrethroid resistant ticks by adult immersion test

Acaricide	Susceptible	Aldama[a]	Mora[b]	Coatzacoalcos[c]
Chlorfenvinphos, 300 ppm	99.99	95.27	42.01	98.19
Coumaphos, 200 ppm	97.94	25.84	34.14	
Flumetrina[d]	100	45.95	0	100
Cypermetrina, 200 ppm	92.76	99.59	0	80.26
Deltametrina, 20 ppm	100	67.79	0	95.56

[a] Aldama strain.
[b] Mora strain.
[c] Coatzacoalcos strain.
[d] Not done.

et al. (1993) treated 8000–12,000 cattle with deltamethrin at 7–14 day intervals and as a result, the numbers of tsetse decreased by ∼90% and the prevalence of trypanosomosis declined from 10.5 to 3.0%. Prior to the tsetse control operation, cattle were treated regularly with dioxathion to control ticks. The change to deltamethrin had no significant effect on tick burdens, but did reduce the prevalence of anaplasmosis (from 21 to 3%) and anaplasmosis-related mortality (3.6–0.4% per year). The authors suggested that the unexpected effect on anaplasmosis was because the immunosuppression produced by chronic infection with trypanosomes was causing patent parasitaemia and disease to emerge in premune carrier animals. Other factors might have played a role in the reduced incidence of anaplasmosis, including reductions in: (1) the numbers of biting flies, which are particularly important in the mechanical transmission of *Anaplasma marginale* when tick numbers are low; and (2) iatrogenic needle transmission resulting from reduced use of trypanocidal drugs.

Results from Zimbabwe provide a contrasting example of tsetse control possibly increasing susceptibility to tick-borne disease. In NE Zimbabwe cattle are treated regularly with deltamethrin to prevent tsetse re-invasion from infested areas of Mozambique. Van den Bossche and Mudenge (1999) found that deltamethrin-treated cattle had a seroprevalence of antibodies against *Babesia bigemina* of only 2% compared to 43% for neighboring Amitraz-treated cattle. *B. bigemina*-naïve sentinel cattle grazed in the tsetse control zone also exhibited low seroprevalences to *B. bigemina* on seven successive 3-monthly sampling occasions. The results are somewhat equivocal for two reasons. First, the relatively high seroprevalence in the Amitraz-treated areas is puzzling, since this acaricide is also effective against *Boophilus*. The authors suggested that the difference was because dipping was carried out more effectively within the tsetse-control area. Second, sentinel cattle were not deployed in the Amitraz-zone and thus there are no data with which to compare the results from the deltamethrin-zone.

Despite these uncertainties, the combination of theoretical and empirical evidence suggests that using pyrethroid-treated cattle to control tsetse can reduce or increase the prevalence of tick-borne diseases. Can strategies to capitalize on the benefits be developed? Tsetse feed selectively on older hosts, and both ticks and tsetse feed at particular sites on the bodies of hosts. *Glossina pallidipes* for instance, feeds largely on the legs of older cattle (Torr and Hargrove, 1998; Torr et al., 2001), whereas adult *Ambylomma* spp. attach to the ventral torso, axillae, scrotum, udder and perineum. Thus, by applying insecticide only to the legs of adult cattle we might control *G. pallidipes* effectively while still allowing young cattle to be exposed to *Amblyomma* and hence develop an immunity to cowdriosis. Research is currently being undertaken in South Africa, Tanzania, Uganda and Zimbabwe to develop application regimes that are effective against various species of tsetse.

3.5. Survey of Australian producers

The results of questions relating to the level of concern about cattle tick control are shown in Table 5, and responses to the question relating to management changes on properties since the diagnosis of amitraz resistance are summarized in Table 6. Farmers were asked if amitraz has been used since resistance diagnosis, and if yes, how consistently and how frequently (Table 7). Farmers identified as continuing to use amitraz were asked to recall the frequency

Table 5
Farmer rated concern for cattle tick control (farmers were asked, "How do you rate your current concern for cattle tick control?")

Rating	Dairy	Beef	Mixed herds	Total
Very concerned	5/12	4/28	3/7	12/47
Concerned	4/12	10/28	3/7	17/47
Unconcerned	3/12	14/28	1/7	18/47

Responses are proportions of all respondents answering that question.

Table 6
Farmer rated concern for cattle tick control (farmers were asked, "Have you made a change to stock numbers or breed since resistance diagnosis as a result of tactic/amitraz failure?")

Farm type	Yes	Percentage	95% CI (%)	Breed change	Change description
Dairy	1/12	8	0–23	0/12	Ceased use of leased land for cattle
Beef	9/29	31	14–48	9/29	To Brahman (7/9) and Droughtmaster (1/9)

Table 7
Amitraz use since resistance diagnosis (farmers were asked, "Have you used tactic/amitraz since resistance diagnosis?"(question 1); "What acaricides/dip did you employ for the last three treatments?"; "How many times have you used tactic/amitraz in the preceding 12 months?")

Farm type	Amitraz used since diagnosis of resistance	Percentage	95% CI (%)	Last three treatments amitraz	Percentage	Number of amitraz treatments in preceding 12 months	Range
Dairy	8/12	66	40–94	2/8	25	4.6	1–17
Beef	18/26	70	52–88	7/18	38	8.1	3–9
All[a]	33/44	75	62–88			8.3	3–20

[a] Includes farms with mixed beef/dairy herds.

of all acaricide applications before and after resistance diagnosis (Table 8). The farmers' approaches to tick control (strategic or timed intervention versus threshold or reactive) before and after the diagnosis of resistance are shown in Table 9. No farmers changed their approach to tick control from strategic to threshold or vice versa. Tables 10–12 summarize the estimated frequency and manner of obtaining and selling cattle.

Table 8
Acaricide treatments before and after resistance diagnosis for farmers continuing to use amitraz

Farm type	Number of years amitraz in use before diagnosis	Range	Applications per year before diagnosis	Range	Applications in calendar year before interview	Range
Dairy	8	1–15	9.4	7–12	5.3	1–7
Beef	12.4	2–30	8.4	3–20	7.8	3–20
All[a]			8.7	3–20	8.3	3–20

[a] Includes farmers with mixed beef/dairy operations.

Table 9
Farmers were asked, “How did you decide when to apply cattle tick treatments before resistance diagnosis?” and “How do you currently decide when to apply cattle tick treatments?”

Farm type	Strategic treatments	Percentage	95% CI	Ticks start to appear	Percentage	95% CI	Animals are visually loaded	Percentage	95% CI
Dairy[a]	3/8	37	4–70	3/8	37	4–70	2/8	25	0–55
Beef[a]	4/24	17	2–32	7/24	29	11–47	13/24	54	34–74

[a] All criteria are represented equally before and after resistance diagnosis.

Table 10
Frequency of cattle purchases (farmers were asked, “Over the 5 years prior to resistance diagnosis how often did you purchase cattle onto your property?”)

Farm type	Never or rarely	Percentage	95% CI	Annually or biannually	Percentage	95% CI	Monthly	Percentage	95% CI	No response
Dairy	5/10	50		3/10	30		1/10	10	0–29	2/10
Beef	10/23			6/23			7/23	30	11–49	6/23
All[a]	15/38			13/38			9/38	24		10/38

[a] Includes farms with mixed beef/dairy herds.

Table 11
Method of purchase and location (farmers were asked, “Over the 5 years prior to resistance diagnosis were any purchases made through dispersal sales, sale yards or from private sales?” “Were any purchases made from the Mt. Larcom area, Gympie area or Wide Bay area?”)

Farm type	Dispersal sale	Percentage	95% CI	Sale yards	Percentage	95% CI	Private sale	Percentage	95% CI
Dairy	5/10	50		5/10	50		8/10	80	
Beef	7/23	30		17/23	74		14/23	61	
All[a]	15/38	40		23/38	61		26/38	68	

[a] Includes farms with mixed beef/dairy herds.

Table 12
Cattle sales (farmers were asked, “Since resistance diagnosis have you sold any cattle through dispersal sales, sale yards or private sales?”)

Farm type	Dispersal sales	Percentage	95% CI	Sale yards	Percentage	95% CI	Private sale	Percentage	No response	95% CI
Dairy	0/10	0		9/10	90		4/10	40	2/10	
Beef	3/23	13		17/23	74		8/23	35	6/23	
All[a]	4/39	10		32/39	82		13/39	33	7/39	

[a] Includes farms with mixed beef/dairy herds.

The level of concern among farmers on farms with resistance to amitraz was surprisingly low given the economic impact that resistance has. Twenty-five percent of dairy farmers and 54% of beef farmers were unconcerned by cattle tick control. The only product available to dairy farmers with resistance to all synthetic pyrethroids (SP) and to amitraz is moxidectin, and dairy cattle in Queensland are pure *Bos taurus*, almost without exception. This was seen in the present study: all dairy farmers who ceased using amitraz are now using moxidectin, whereas beef farmers have commonly adopted one of three alternative acaricides: fluazuron, cypermethrin/chlorfenvinphos or one of the macrocyclic lactones. Further, about one third of beef producers changed to more resistant cattle breeds after the diagnosis of amitraz resistance, but no dairy farmers changed breeds.

Changes to the farming operation, reported as a direct consequence of amitraz resistance, were more frequent for beef farmers than dairy farmers. All changes on beef farms were changes to herd breed composition to improve host resistance to cattle ticks. For over 20 years, improving host resistance by increasing *B indicus* content has been the major method of non-chemical control of cattle ticks in the northern Australian beef industry (Sutherst and Utech, 1981). The only dairy farm that changed any of its management simply ceased to graze stock on one rented dry cow run where resistance was very evident. Only one dairy farmer of 21 attempted to select for naturally tick resistant cattle.

Amitraz was employed as an acaricide for an average of 12 years (maximum 30 years) prior to resistance diagnosis. Despite the presence of laboratory confirmed amitraz resistance and dip failure in the field, the majority of farmers (67% of dairy farmers and 69% of beef farmers) report ongoing use of amitraz. Our results show that the criteria for farmers' decisions about the timing of acaricide applications did not change following the diagnosis of resistance. The majority of farmers (63% of dairy farmers and 83% of beef farmers) continued to apply acaricides at irregular intervals when ticks were seen. After the diagnosis of resistance, the number of applications of acaricide in each year remained comparable to that before the diagnosis of resistance.

Resistance to acaricides is clearly likely to spread with the movement of infested cattle within the infested area of Queensland. The present survey suggests that the majority of dairy and beef producers with amitraz resistance had purchased cattle within the 5 years before diagnosis. Thirty percent of beef producers had purchased cattle monthly or more often. Eighty-two percent of all producers had sold cattle through public sale yards since the diagnosis of resistance, likely resulting in further dispersal of amitraz resistant ticks.

4. Conclusions

4.1. Mechanisms of resistance

There is a mixture of resistance mechanisms found for each class of acaricide studied in Mexican *B. microplus.* These include metabolic detoxification involving *cytP450* and esterases for SP and PY resistance, respectively, and target sight alterations for every class of acaricide examined. These studies have lead to the development of several molecular based techniques that rapidly detect OP and SP resistance. Currently, there are no rapid molecular based methods to detect AM resistance.

The role of AChE in *B. microplus* OP resistance mechanisms remains to be clarified. Although biochemical evidence shows AChE from OP resistant tick populations has different inhibition kinetic parameters from OP sensitive populations, the molecular mechanism of target site insensitivity remains to be identified. Much more is known about OP and SP resistance than amitraz resistance in *B. microplus*. Our studies show that esterases are involved, but a search for a target sight mutation is ongoing.

4.2. Molecular techniques

Future research will better characterize the different types of resistance found in Boophilus and measure the geographical extent of each resistant population. Three molecular assays for pyrethroid resistance in *B. microplus* have been developed. A PCR assay diagnoses target site-based pyrethroid resistance, which appears to play a major role in several populations from Mexico. An HPLC-based assay detects the major metabolite of esterase-based permethrin-hydrolysis, an important resistance mechanism in at least one population of resistant Mexican ticks. A PCR assay detects a mutation in a permethrin-hydrolyzing esterase, CzEst9. The mutation plays only a minor role in the hydrolytic capacity of CzEst9, which seems to confer resistance by overexpression. Development of rapid molecular-based techniques to detect resistance will allow the personnel of the Cattle Fever Tick Eradication Program (CFTEP) to have a better understanding of the *B. microplus* populations introduced into the United States. This understanding will aid in the quick and efficient elimination of these populations.

4.3. Resistance versus enzootic stability

The development of acaricide resistance of *B. microplus* in Mexico to OP and amidines has been slow in comparison with the SP resistance which spread rapidly. The OP "Tuxpan" *B. microplus* strain had been under acaricide selection pressure and needed more than seven generations to obtain a homozygous resistant tick colony from the original strain. However, in the case of an SP resistance strain collected from the field, it was found to be almost homozygous resistant. Only in the case of SP resistant *B. microplus* ticks has there been reported babesiosis outbreaks with 3% mortality rate or higher in adult animals and 5% morbidity rate -in regions which were considered to be enzootically stabile. We assumed that bovine babesiosis in Mexico, in some cases *B. microplus* SP resistance outbreaks, were due to the tick population increases and the presence of low herd immunity, but this situation does not occur in the case of OP or amidine resistance since the development is slow and herd immunity is maintained.

4.4. Effects of fly control on ticks and tick-borne disease

Simple analytical models of tick- and tsetse-borne diseases suggest that reducing the abundance of ticks, by treating cattle with pyrethroids for example, can have a variety of effects on tick-borne diseases. In the worst-case scenario, the models suggest that treating cattle might not only have no impact on trypanosomosis but could increase the incidence of tick-borne disease. In the best case, treatment could reduce the incidence of both trypanosomosis and

tick-borne diseases. The predicted outcome varies according to epidemiological starting conditions and the spatial and temporal extent of the tsetse control operation. Examples from several countries provide evidence to support some of these theoretical predictions.

4.5. Management strategies for resistant ticks

The problem of cattle tick control most acutely impacts on dairy farmers due to the low host resistance of Holstein Friesians and limited cost effective alternatives to the common practice of frequent amitraz dipping. Beef farmers have a greater choice of acaricides available for use and a greater choice of resistant cattle breeds to stock with. As a result, changes in beef herd composition following resistance detection were common and are ongoing. In contrast to patterns of acaricide use seen following the detection of resistance to other classes of acaricides, amitraz continues to be used regularly after emergence of resistance. This might suggest that resistance to amitraz incurs a significant fitness cost. Frequent movement of cattle on and off farms with amitraz resistance, together with limited evidence of a fitness cost associated with amitraz resistance , suggests that attention to improved biosecurity might help to limit the spread of resistance to this very useful acaricide.

4.6. Overview

We have presented some of the factors that influence the prevalence of acaricide resistance and tick-borne diseases. Following the eradication of a tick vector and an associated tick-borne disease, herd immunity is lost and vigilance to prevent the reintroduction of the tick vector must be maintained. Protecting borders from entry of ticks on cattle is a difficult task, and it is important to be certain that control techniques will be effective when tick infestations are discovered. We have reported several studies on the detection of acaricide resistance in tick populations in Mexico, and described the mechanisms of this resistance, as well as assays needed, for the rapid diagnosis of resistant tick strains. In areas where ticks and tick-borne diseases currently exist, the need for tick control and the need for exposure of young animals to ticks to maintain herd immunity must be balanced. Resistance in tick populations may actually aid in maintaining this balance, particularly when producers continue to use the same acaricide after resistance has been identified. The use of pesticides for the control of pests of cattle other than ticks can affect the incidence of tick resistance and tick-borne diseases. An example of how this can be countered exists in tsetse control programs that target insecticide application to specific tsetse feeding sites on adult cattle while allowing young cattle to become immunized by exposure to ticks.

Acknowledgements

Sincere thanks to Kevin Duff, Malcolm McLeod, and Vern Doyle from DPI Queensland for the provision of collated property information and records of resistance diagnosis. We thank Charlene Wilson and Christine Hagius for technical assistance. Published with

approval of the Director of the Louisiana Agricultural Experiment Station as Manuscript no. 03-26-1634.

References

Bauer, B., Kabore, I., Liebisch, A., Meyer, F., Petrich-Bauer, J., 1992. Simultaneous control of ticks and tsetse flies in Satiri, Burkina Faso, by the use of flumethrin pour-on for cattle. Trop. Med. Parasitol. 42, 41–46.

Baxter, G.D., Barker, S.C., 1998. Acetylcholinesterase cDNA of the cattle tick, *Boophilus microplus*: characterization and role in organophosphate resistance. Insect Biochem. Mol. Biol. 28, 581–589.

Benavides, O.E., 1985. Consideraciones con relación a la epizootiología de Anaplasmosis y Babesiosis en los bovinos. Rev. ACOVEZ 9, 4–11.

Dransfield, R.D., Brightwell, R., Williams, B., 1990. Control of tsetse fly (Diptera: Glossinidae) populations using traps at Nguruman, south-west Kenya. Bull. Entomol. Res. 80, 265–276.

FAO, 1984. Acaricide resistance. In: Ticks and Tick-borne Disease Control. A Practical Field Manual, vol. 1. Tick Control, FAO, Rome, pp. 246–299.

Fox, R.G.R., Mmbando, S.O., Fox, M.S., Wilson, A., 1993. Effect on herd health and productivity of controlling tsetse and trypanosomiasis by applying deltamethrin to cattle. Trop. Anim. Health Product. 25, 203–214.

Guerrero, F.D., Davey, R.B., Miller, R.J., 2001. Use of an allele-specific polymerase chain reaction assay to genotype pyrethroid resistant strains of *Boophilus microplus* (Acari: Ixodidae). J. Med. Entomol. 38, 44–50.

Guerrero, F.D., Li, A.Y., Hernandez, R., 2002. Molecular diagnosis of pyrethroid resistance in Mexican strains of *Boophilus microplus* (Acari: Ixodidae). J. Med. Entomol. 39, 770–776.

Hargrove, J.W., 2000. A theoretical study of the invasion of cleared areas by tsetse flies (Diptera: Glossinidae). Bull. Entomol. Res. 90, 201–209.

He, H., Chen, A.C., Davey, R.B., Ivie, G.W., George, J.E., 1999. Identification of a point mutation in the para-type sodium channel gene from a pyrethroid-resistant cattle tick. Biochem. Biophys. Res. Com. 261, 558–561.

Hernandez, R., He, H., Chen, A.C., Ivie, G.W., George, J.E., Wagner, G.G., 1999. Cloning and sequencing of a putative acetylcholinesterase cDNA from *Boophilus microplus* (Acari: Ixodidae). J. Med. Entomol. 36, 764–770.

Hernandez, R., He, H., Chen, A.C., Waghela, S.D., Ivie, G.W., George, J.E., Wagner, G.G., 2000. Identification of a point mutation in an esterase gene in different populations of the southern cattle tick, *Boophilus microplus*. Insect Biochem. Mol. Biol. 30, 969–977.

Hernandez, R., Guerrero, F.D., George, J.E., Wagner, G.G., 2002. Allele frequency and gene expression of a putative carboxylesterase-encoding gene in a pyrethroid resistant strain of the tick, *Boophilus microplus*. Insect Biochem. Mol. Biol. 32, 1009–1016.

Jamroz, R.C., Guerrero, F.D., Pruett, J.H., Oehler, D.D., Miller, R.J., 2000. Molecular and biochemical survey of acaricide resistance mechanisms in larvae from Mexican strains of the southern cattle tick, *Boophilus microplus*. J. Insect Physiol. 46, 685–695.

Jordan, A.M., 1986. Trypanosomiasis Control and African Rural Development. Longman, London/New York.

Li, A.Y., Davey, R.B., Miller, R.J., George, J.E., 2003. Resistance to coumaphos and diazinon in *Boophilus microplus* (Acari: Ixodidae) and evidence for the involvement of an oxidative detoxification mechanism. J. Med. Ent. 40, 482–490.

Li, A.Y., Davey, R.B., Miller, R.J., George, J.E., 2004. Detection of amitraz resistance in the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae). J. Med. Ent. 41, 193–200.

Miller, R.J., Davey, R.B., George, J.E., 1999. Characterization of pyrethroid resistance and susceptibility to coumaphos in Mexican *Boophilus microplus* (Acari: Ixodidae). J. Med. Entomol. 36, 533–538.

Pruett, J.H., 2002. Comparative inhibition kinetics for acetylcholinesterases extracted from organophosphate resistant and susceptible strains of *Boophilus microplus* (Acari: Ixodidae). J. Econ. Entomol. 95, 1239–1244.

Pruett, J.H., Guerrero, F.D., Hernandez, R., 2002. Isolation and identification of an esterase from a Mexican strain of *Boophilus microplus* (Acari: Ixodidae). J. Econ. Entomol. 95, 1001–1007.

Willemse, S.S., 1991. Ecología de las garrapatas *Boophilus*: perspectivas de un panorama. In: II Seminario Internacional de Parasitología Animal Garrapatas y Enfermedades que Transmiten, vol. 75, México, pp. 19–30.

Sutherst, Utech, K.B.W., 1981. Controlling livestock parasites with host resitance. In: Pimentel, D. (Ed.), CRC Handbook of Pest Management in Agriculture, vol 2. CRC Press Inc., Boca Raton, FL.

Torr, S.J., Hargrove, J.W., 1998. Factors effecting the landing and feeding responses of the tsetse fly *Glossina pallidipes* to a stationary ox. Med. Vet. Entomol. 12, 196–207.

Torr, S.J., Wilson, P.J., Schofield, S., Mangwiro, T.N.C., Akber, S., White, B.N., 2001. Application of DNA markers to identify the individual-specific hosts of tsetse feeding on cattle. Med. Vet. Entomol. 15, 78–86.

Thompson, J.W., Mitchell, M., Rees, R.B., Shereni, W., Schoenfeld, A.H., Wilson, A., 1991. Studies on the efficacy of deltamethrin applied to cattle for the control of tsetse flies (*Glossina* spp.) in southern Africa. Trop. Anim. Health Product. 23, 221–226.

Vale, G.A., 1993. Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. J. Med. Entomol. 30, 831–842.

Van den Bossche, P., Mudenge, D., 1999. The effect of short-interval deltamethrin applications to control tsetse on the seroprevalncen of babesiosis in cattle. Trop. Anim. Health Product. 31, 215–222.

Willemse, L., 1991. A trial of odour-baited targets to control the tsetse fly, *Glossina morsitans centralis* (Diptera: Glossinidae) in west Zambia. Bull. Entomol. Res. 81, 651–657.